

35. The method of Claim 34 wherein said tag comprises a fluorescent group.
36. The method of Claim 35 wherein said fluorescent group is selected from the group consisting of 5-carboxyfluorescein, 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein, N,N,N',N'-tetramethyl-6-carboxyrhodamine, 6-carboxy-X-rhodamine, Fluorescein, Rhodamine, BODIPY-TR-X, and Cascade Blue, and Alexa 350.
37. The method of Claim 34 wherein said tag absorbs at a wavelength different from said polynucleotide.
38. The method of claim 34 wherein said medium comprises polymer beads having an average diameter of 0.5 to 100 microns and having a surface composition that is either unsubstituted or essentially completely substituted with a moiety selected from the group consisting of hydrocarbon having from 1 to 1,000,000 carbons, and hydrocarbon polymer having from 1 to 1,000,000 carbons.
39. The method of claim 34 wherein said medium comprises beads having an average diameter of 0.5 to 100 microns, the beads comprising nonporous particles coated with a hydrocarbon or non-polar hydrocarbon substituted polymer, or particles having substantially all polar groups reacted with a non-polar hydrocarbon or substituted hydrocarbon group, wherein said particles are a member selected from the group consisting of silica, silica carbide, silica nitride, titanium oxide, aluminum oxide, zirconium oxide, carbon, insoluble polysaccharide, and diatomaceous earth.
40. The method of claim 34 wherein said tagged polynucleotide comprises a PCR amplification product obtained by providing a PCR primer having a covalently bound tag during a PCR amplification wherein said tag is incorporated into said PCR amplification product.
41. The method of claim 34 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.
42. A method for enhancing the detection of a polynucleotide separated by reversed phase ion pairing chromatography, said method comprising:
- a) covalently attaching a chemical tag to said polynucleotide to form a tagged polynucleotide.

- b) applying said tagged polynucleotide to a separation bed of separation beads having non-polar surfaces, wherein said separation beads are substantially free of multivalent cations capable of interfering with polynucleotide separation,
- c) eluting said tagged polynucleotide from said particles with a mobile phase containing a counterion agent and an organic solvent, and
- d) detecting said tagged polynucleotide, wherein steps (b) and (c) are performed in a system for separating a mixture of polynucleotide fragments comprising a chromatographic column having two ends, said column containing said separation bed of separation beads having non-polar surfaces held in the column between porous frits positioned at each end thereof, said column having an inlet, an injection valve in communication with said inlet through a flow path therebetween, mobile phase supply means in communication with said injection valve through at least one flow path therebetween, and multivalent cation capture resin, selected from the group consisting of cation exchange resin and chelating resin, positioned in said flow path, said multivalent cation capture resin being capable of removing multivalent cations from aqueous solutions, whereby any multivalent cation contaminants in said flow path are removed before said contaminants contact the separation bed.

43. A method for enhancing the detection of a polynucleotide separated by reversed phase ion pairing chromatography, said method comprising:

- a) covalently attaching a chemical tag to said polynucleotide to form a tagged polynucleotide,
- b) applying said tagged polynucleotide to a separation bed of separation beads having non-polar surfaces, wherein said separation beads are substantially free of multivalent cations capable of interfering with polynucleotide separation,
- c) eluting said tagged polynucleotide from said particles with a mobile phase containing a counterion agent and an organic solvent,
- d) detecting said tagged polynucleotide, wherein steps (b) and (c) are performed in a system for separating a mixture of polynucleotide fragments the

system comprising a chromatographic column having two ends, said column containing a separation bed of separation beads having non-polar surfaces held in the column between porous frits positioned at each end thereof, said column having an inlet, an injection valve in communication with said inlet through a conduit, eluant supply means in communication with said injection valve through at least one conduit, wherein said porous frits, chromatographic column, injection valve, eluant supply means, and conduits have process solution-contacting surfaces which contact process solutions held therein or flowing therethrough, and wherein the process solution-contacting surfaces of said porous frits are material which does not release multivalent cations into aqueous solutions flowing therethrough.

44. The method of claim 43 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.
45. A method for increasing the retention time of a polynucleotide separated by reversed phase ion pairing chromatography, said method comprising:
- a) covalently attaching a chemical tag to said polynucleotide to form a tagged polynucleotide,
  - b) applying said tagged polynucleotide to a separation medium having a non-polar surface, wherein said separation medium is substantially free of multivalent cations capable of interfering with polynucleotide separation,
  - c) eluting said tagged polynucleotide from said surface with a mobile phase containing a counterion agent and an organic solvent,
  - d) detecting said tagged polynucleotide.
46. The method of claim 45 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.
47. The method of Claim 45 wherein said tag comprises a hydrocarbon group, wherein said hydrocarbon group is selected from the group consisting of alkyl, cycloalkyl, aryl and arylalkyl groups.

48. A method for enhancing the detection of a polynucleotide separated by reversed phase ion pairing chromatography, said method comprising:
- a) contacting said polynucleotide with a reversible DNA-binding dye to form a complex between said polynucleotide and said reversible DNA-binding dye,
  - b) applying said complex to a separation medium having a non-polar surface, wherein said separation medium is substantially free of multivalent cations capable of interfering with polynucleotide separation,
  - c) eluting said complex from said surface with a mobile phase containing a counterion agent and an organic solvent, and
  - d) detecting said complex.
49. The method of claim 48 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.
50. The method of claim 48 in which said reversible DNA-binding dye is selected from the group consisting of DNA intercalator dye and DNA groove binding dye.
51. The method of claim 50 in which said reversible DNA-binding dye is selected from the group consisting of PICO GREEN, ethidium bromide, propidium iodide, Acridine orange, 7-aminoactinomycin D, cyanine dye, Bisbenzimidazole, Benzoxanthene yellow, Netropsin, Indole dye, Imidazole dye, and Actinomycin D.
52. A method for the detection of a mutation in a sample double stranded DNA fragment, said method comprising:
- a) covalently attaching a chemical tag to at least one of said sample DNA fragment or a corresponding wild type fragment to form a tagged polynucleotide,
  - b) hybridizing said sample DNA fragment with said corresponding wild type DNA fragment to form a mixture of homoduplexes and heteroduplexes if a mutation is present in said sample DNA fragment,
  - c) applying the product of step (b) to a separation medium having a non-polar separation surface, wherein said separation medium is substantially free

of multivalent cations capable of interfering with polynucleotide separation,

d) eluting said mixture with a mobile phase containing a counterion agent and an organic solvent where said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where said eluting results in the separation of said heteroduplexes from said homoduplexes, and

e) monitoring said mobile phase during said eluting for the presence of tagged heteroduplex, wherein the presence of tagged heteroduplex indicates the presence of said mutation.

53. The method of claim 52 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.

54. The method of claim 52 in which a different uniquely detectable tag is covalently attached to each strand of said sample DNA.

55. The method of claim 52 in which a different uniquely detectable chemical tag is covalently attached to each strand of said wild type fragment.

56. The method of claim 52 in which said wild type fragment in step (b) is tagged and the amount of said wild type fragment is added in excess of said sample DNA.

57. A method for increasing the melting temperature of a double stranded DNA as determined by temperature titration by reversed phase ion pairing chromatography, said method comprising:

covalently binding a non-polar chemical tag to said DNA, to form a tagged polynucleotide, prior to said temperature titration,

wherein said temperature titration is performed by (a) applying the tagged polynucleotide to a separation medium having a non-polar separation surface, wherein said surface is substantially free of multivalent cations capable of interfering with polynucleotide separation, (b) eluting the tagged polynucleotide from the surface with a mobile phase containing a counterion agent and an organic solvent, and (c) detecting the tagged polynucleotide, wherein steps (a) and (b) are performed at a plurality of temperatures above and below the melting temperature.

58. The method of claim 57 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.
59. The method of claim 57 wherein said non-polar chemical tag comprises a hydrocarbon group, wherein said hydrocarbon group is selected from the group consisting of alkyl, cycloalkyl, aryl and arylalkyl groups.
60. The method of claim 57 wherein said non-polar tag comprises a fluorescent group.
61. The method of claim 57 wherein said non-polar tag is bound at an end of said DNA.
62. A method for detecting a covalently tagged polynucleotide separated by reversed phase ion pairing chromatography, said method comprising:
- a) applying said tagged polynucleotide to a separation medium having a non-polar surface, wherein said separation medium is substantially free of multivalent cations capable of interfering with polynucleotide separation,
  - b) eluting said tagged polynucleotide from said surface with a mobile phase containing a counterion agent and an organic solvent, and
  - c) detecting said tagged polynucleotide.
63. The method of claim 62 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.
64. The method of Claim 62 wherein said tag comprises a fluorescent group.
65. The method of Claim 62 wherein said tag absorbs at a wavelength different from said polynucleotide.
66. A method for detecting a complex comprising a polynucleotide bound to a reversible DNA-binding dye, as separated by reversed phase ion pairing chromatography, said method comprising:
- a) applying said complex to a separation medium having a non-polar surface, wherein said separation medium is substantially free of multivalent cations capable of interfering with polynucleotide separation,
  - b) eluting said complex from said surface with a mobile phase containing a counterion agent and an organic solvent, and
  - c) detecting said complex.

67. The method of claim 66 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.
68. A method for enhancing the detection of a polynucleotide separated by reversed phase ion pairing chromatography, said method comprising:
- a) covalently attaching a chemical tag to said polynucleotide to form a tagged polynucleotide,
  - b) applying said tagged polynucleotide to a separation medium having a non-polar surface,
  - c) eluting said tagged polynucleotide from said surface with a mobile phase containing a counterion agent and an organic solvent, and
  - d) detecting said tagged polynucleotide, wherein said separation medium is substantially free of multivalent cations capable of interfering with polynucleotide separation, wherein said medium comprises a polymeric monolith.
69. The method of claim 68 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.
70. A method for enhancing the detection of a polynucleotide separated by reversed phase ion pairing chromatography, said method comprising:
- a) covalently attaching a chemical tag to said polynucleotide to form a tagged polynucleotide,
  - b) applying said tagged polynucleotide to a separation medium having a non-polar surface,
  - c) eluting said tagged polynucleotide from said surface with a mobile phase containing a counterion agent and an organic solvent, and
  - d) detecting said tagged polynucleotide, wherein said separation medium is substantially free of multivalent cations capable of interfering with polynucleotide separation, wherein said medium comprises a derivatized silica gel monolith.

71. The method of claim 70 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.
72. A method for increasing the retention time of a polynucleotide separated by reversed phase ion pairing chromatography, said method comprising:
- a) covalently attaching a chemical tag to said polynucleotide to form a tagged polynucleotide,
  - b) applying said tagged polynucleotide to a separation medium having a non-polar surface,
  - c) eluting said tagged polynucleotide from said surface with a mobile phase containing a counterion agent and an organic solvent,
  - d) detecting said tagged polynucleotide, wherein said separation medium is substantially free of multivalent cations capable of interfering with polynucleotide separation, wherein said chemical tag is non-polar, wherein said tag comprises a hydrocarbon group, wherein said hydrocarbon group is selected from the group consisting of alkyl, cycloalkyl, aryl and arylalkyl groups.
73. The method of claim 72 wherein said multivalent cations comprise a member selected from the group consisting of Fe(III), Cr(III), colloidal metal contaminants and mixture of one or more thereof.

#### **REMARKS**

The above amendment identifies the relationship of this application to copending applications.

The claims have been amended, without prejudice, to more particularly define what the Applicants regard as the invention. These amendments do not introduce new matter and are fully supported by the specification.

It is believed that this amendment does not introduce any new matter.

An early examination and allowance of the above-identified application is respectfully requested. The Examiner is respectfully requested to telephone the undersigned attorney of record if anything further is required to place this application into a condition for allowance.